APPENDIX B

CLAIMS PENDING IN USSN 09/854,417 WITH ENTRY OF THIS AMENDMENT

1. (AMEDED) A method for detecting a nucleic acid, the method comprising:

contacting a first nucleic acid to a second nucleic acid, which second nucleic acid comprises a neutral or positively charged fluorescent label when hybridized to the first nucleic acid; and,

detecting fluorescence polarization of the resulting mixture of first and second nucleic acids.

2. A method for detecting a nucleic acid, the method comprising:
contacting a first nucleic acid to a second nucleic acid, which second nucleic acid comprises a neutral or positively charged fluorescent label; and,detecting fluorescence polarization of the resulting mixture of first and second nucleic acids, wherein the fluorescence polarization is increased by less than about 50% by the addition of polylysine to the first and second nucleic acid.

3. The method of claim 1, wherein the mixture of first and second nucleic acids is present in a composition which is substantially free of polyion.

4. The method of claim 3, wherein the composition comprises less than 1μM polyion.

5. The method of claim 1, wherein a rotational diffusion rate of a duplex of the first and second nucleic acid is less than a rotational diffusion rate of the first or second nucleic acid.

6. The method of claim 5, wherein the fluorescence polarization of unduplexed first or second nucleic acid is at least 50% different than the fluorescence polarization of the duplexed nucleic acid.

7. The method of claim 1, wherein the first or second nucleic acid comprises one or more of: DNA, RNA, LNA, a DNA analogue, an RNA analogue or a PNA.

8. The method of claim 1, wherein one or more of the nucleic acids is nuclease resistant.

9. The method of claim 1, wherein the fluorescent label comprises rhodamine or BODIPY.

10. The method of claim 1, wherein the first nucleic acid is a DNA and the second nucleic acid is a PNA which comprises a rhodamine label.

11. The method of claim 1, wherein the first or second nucleic acids comprise at least a region which is single-stranded.

12. The method of claim 11, wherein the first and second nucleic acid are perfectly complementary.

13. The method of claim 11, wherein the first and second nucleic acid comprise at least one non-complementary nucleotide when aligned for maximum complementarity.

14. The method of claim 11, further comprising determining from the fluorescence polarization detection whether the first and second nucleic acids are duplexed.

15. The method of claim 11, further comprising determining the extent to which the first and second nucleic acids are duplexed from the fluorescence polarization detection.

16. The method of claim 1, wherein the first and second nucleic acids hybridize in solution prior to detection of fluorescence polarization.

17. The method of claim 16, comprising comparing the detected fluorescence polarization to a fluorescence polarization measurement of either the first or the second nucleic acid alone in solution.

18. The method of claim 16, comprising comparing the detected fluorescence polarization to a fluorescence polarization measurement of either the first or the second nucleic acid hybridized to a third nucleic acid.

19. The method of claim 18, wherein the third nucleic acid is perfectly complementary to either the first or the second nucleic acid.

20. The method of claim 18, wherein the third nucleic acid is not perfectly complementary to either the first or the second nucleic acid.

21. The method of claim 18, wherein the third nucleic acid is unrelated in sequence to either the first or the second nucleic acid.

22. The method of claim 16, comprising detecting fluorescence polarization during hybridization of the first and second nucleic acid.

23. The method of claim 22, further comprising determining the fluorescence polarization as a function of time during hybridization of the first and second nucleic acid.

24. The method of claim 23, further comprising plotting a histogram of the fluorescence polarization as a function of time.

25. (AMENDED) A method of identifying the presence of a subsequence of nucleotides in a target nucleic acid, the method comprising:

contacting the target nucleic acid sequence with a labeled nucleic acid probe, which labeled nucleic acid probe comprises a neutral or positively charged label comprising a fluorophore to form a first reaction mixture; and,

detecting the level of fluorescence polarization of the first reaction mixture, wherein the probe comprises the labeled fluorophore when hybridized to the target nucleic acid.

26. The method of claim 25, wherein the target nucleic acid sequence comprises at least one locus for a single nucleotide polymorphism.

27. The method of claim 26, wherein the nucleic acid probe is complementary to one allele of the single nucleotide polymorphism in the target nucleic acid sequence.

28. The method of claim 25, comprising contacting a plurality of additional target nucleic acids with a plurality of additional labeled nucleic acid probes, which additional labeled nucleic acid probes individually comprise a neutral or positively charged label comprising a fluorophore to form a plurality of additional reaction mixtures; and,

detecting the level of fluorescence polarization of the plurality of additional reaction mixtures.

29. The method of claim 28, wherein the plurality of additional target nucleic acids individually comprise at least one locus for a single nucleotide polymorphism.

30. The method of claim 29, wherein the plurality of additional nucleic acid probes are individually complementary to at least one allele of each of the single nucleotide polymorphisms in the plurality of target nucleic acid sequences.

31. The method of claim 30, wherein the plurality of additional target nucleic acids are derived from a single species, variety, cultivar, cell, virus, or organism.

32. The method of claim 31, wherein identification of the single nucleotide polymorphisms provides a single nucleotide polymorphism genotype for the species, variety, cultivar, cell, virus or organism.

33. The method of claim 25, wherein the fluorescence polarization is increased by less than about 50% by the addition of polylysine to the target and probe nucleic acids.

34. The method of claim 25, wherein the target and probe nucleic acids are present in a composition which is substantially free of polyion.

35. The method of claim 34, wherein the composition comprises less than 1 μM polyion.

36. The method of claim 25, wherein a rotational diffusion rate of a duplex of the target and probe nucleic acids is less than a rotational diffusion rate of the target or probe nucleic acids.

37. The method of claim 36, wherein the fluorescence polarization of the probe which is duplexed to the target is at least 50% different than the fluorescence polarization of the probe when not duplexed to the target.

38. The method of claim 25, wherein the target or probe nucleic acids comprise one or more of: DNA, RNA, LNA, a DNA analogue, an RNA analogue or a PNA.